

1                   --45. The partial cDNA sequence of conductine of claim 40, of the  
2 nucleotide sequences 2561 to 2713 (gene section of the disheveled homology  
3 region) of Seq. ID No. 10, and Fig. 2.--

1 *AM*                   --46. A gene therapy process for tumor diseases, which comprises  
2 *CH* constructing a vector with the conductine gene of claim 33, and restoring conductine  
3 in cells of a patient in need therefor by carrying out a gene transfer in the body of  
4 said patient.--

REMARKS

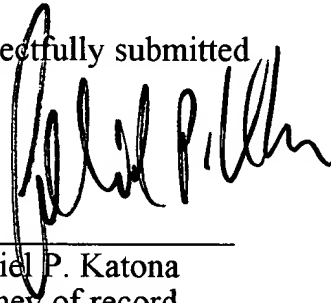
*Add  
Dz*                   Claims 24-46 are in the application.

Favorable action is respectfully urged.

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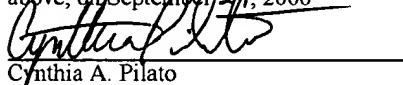
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Respectfully submitted



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I hereby certify that this correspondence is deposited with the U.S. Postal Service as first class mail, addressed as  
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spelling is conductine

0107-026P

**Conductine Protein and a Related Agent  
For Diagnosing and Treating Tumor Illnesses**

Field of the invention

The invention relates to a new way of combating tumor diseases by utilizing molecular biological relationships of the formation of tumors. In particular, it relates to a material for diagnosing tumor diseases and, <sup>new agent</sup> based on this, a material <sup>based on the new agent</sup> for the treatment <sup>of such diseases. The invention also relates to</sup> of the new protein, conductine, its mutants and variations as well as to parts thereof, to the analogous cDNA sequences and to their use in the gene-therapeutic and pharmacological methods. Areas of the application are medicine and the pharmaceutical industry.

Background

Cadherines and catenines form cell adhesion complexes, which are responsible in numerous tissues for the adhesion of cells to one another. The cadherines are trans-membrane proteins and produce the direct contact between adjacent cells.  $\alpha$ ,  $\beta$  and  $\gamma$ -catenine are cytoplasmic components, which connect the cadherines with the actin cytoskeleton. Aside from their function in cell adhesion, the catenines also play a decisive role in signal transduction processes. b-Catenine in vertebrates and the homologous, segment polarity gene product, armadillo in drosophila, are stabilized by the Wnt/wingless signal path (Nusse, R., Cell 89, 321 – 323, 1997). This leads to an increase in the cytoplasmic fraction of these proteins, which is not bound to cadherine, which thereupon could interact with HMG transcription factors of the LEF-1/TCF families. As a result,  $\beta$ -catenine/armadillo is transported into the cell nucleus where, together with the LEF/TCF proteins, it binds to the DNA and activates certain genes (Behrens, J. et al., Nature 382, 638 – 642, 1996).

Brief description of the drawing

The invention is disclosed below with reference being had to the drawing, wherein

15840

Fig. 1 is the amino acid sequence of conductine;

Fig. 2 is the nucleotide sequence of conductine;

Fig. 3 is the gene comparison sequence and the nucleotide sequence; and

Fig. 4 is a showing of of interaction stuidies in the 2-hybrid system.

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This signal path also plays an important role in the formation of tumors. In epithelial cells of the colon, the cytoplasmic pool of  $\beta$ -catenine is strictly regulated by the tumor suppressor gene product APC (Adenomatosis Polyposis Coli). Mutations of APC, such as those occurring in 80% of all colon cancers, lead to shortened forms of the APC protein, which are no longer able to destabilize  $\beta$ -catenine. As a result, permanent complexes of  $\beta$ -catenine with the HMG transcription factor TCF-4, which are <sup>asserted to be</sup> responsible for the transformation of the cells, are found in these tumors. This theory is supported by the recent finding that, in tumors in which APC is not changed, mutations of  $\beta$ -catenine occur. These also lead to cytoplasmic stabilization of  $\beta$ -catenine and to an association with LEF-1/TCF factors (Morin, P.J. et al., Science 275, 1787 – 1790).

*insect*  
Description of the invention

It is an object of the present <sup>to</sup> invention has the goal of finding a new way for preventing the formation of tumors. It is based on the objective of finding a method for controlling the regulation of  $\beta$ -catenine in cells of the body.

It is an <sup>to identify</sup> object of the invention is a new protein which binds to  $\beta$ -catenine and leads to its cytoplasmic breakdown. This protein has the amino acid sequence <sup>shown in</sup> of Figure 1 and <sup>we gave it</sup> was given the name of conductine.

Glycogen synthase kinase 3 $\beta$   
(GSK3 $\beta$ )  
The invention is based on our own realization that conductine binds to APC fragments over a  $\beta$ -catenine binding domain at  $\beta$ -catenine, over a GSK3 $\beta$  binding domain at GSK3 $\beta$  and over a so-called (RGS) domain (regulator of G-protein signaling). As a result, there is cytoplasmic degradation of  $\beta$ -catenine and in vertebrates, blockage of the Wnt/wingless signal path. <sup>establishes</sup> With that, it is clear that conductine is an important regulator of the  $\beta$ -catenine function and in interaction with APC contributes to the suppression of tumors.

which is a part of conductin and interacts with the tumor suppressor protein APC.

Thus

Furthermore, as a consequence, the invention relates to a material for diagnosing tumor diseases, which is characterized in that the presence and the amount of conductine, its mutants and variations or its parts <sup>is</sup> detected in cells of the body. This detection can be carried out on the protein level with specific antibodies, <sup>particularly</sup> especially with monoclonal antibodies.

Pursuant to the <sup>present</sup> invention, the diagnosis of tumor diseases can also be carried out on the gene level. For this purpose,

- the gene, which codes for conductine, its mutants and variations or parts thereof and/or
- mRNA sequences, which are read by these genes, are detected with selected <sup>oligonucleotide</sup> primers and cDNA probes, which are derived from the gene sequence of ~~the~~ <sup>of the present invention</sup> conductine and mutations.

The ~~inventive~~ <sup>present</sup> material for the treatment of tumor diseases contains substances which activate ~~the~~ <sup>reactivate</sup> the action of ~~the~~ <sup>conductine</sup> in the body. Above all, these are materials, which activate the gene promoter of ~~conductine~~ <sup>conducting</sup> or materials, which increase the stability of the mRNA sequences derived from the ~~conductine~~ <sup>conducting</sup> genes. Pursuant to the invention, the main objective of all of these materials <sup>to</sup> ~~consists of~~ <sup>is</sup> increasing the activity of the ~~conductine~~ <sup>conducting</sup> in the cells of the body. For this purpose, substances of low molecular weight, for example, come into consideration, which are found, for example, by high throughput number screening.

The <sup>present</sup> invention also <sup>includes</sup> ~~comprises~~ gene therapeutic materials, containing genes, which code for ~~conductine~~ <sup>conducting</sup>, its mutants and variations or parts thereof, or mRNA sequences, which are read by these genes.

High throughput screening can be performed by analyzing <sup>low</sup> molecular weight substances for their ability to stimulate the activity of the ~~conductin~~ <sup>conductin</sup> promoter or the expression of the ~~conductin~~ <sup>conductin</sup> mRNA/protein after treatment of cultured cells. Alternatively, substances can be screened for active phosphorylation of  $\beta$ -catenin by conductin <sup>in vitro</sup> kinase reactions.

The present invention also relates to a

~~gene~~ gene therapy process for tumor diseases, which comprises constructing a vector with the conductive gene of ~~claim 23~~, and restoring conductive in cells of a patient in need thereof by carrying out a gene transfer in the body of ~~the~~ <sup>the</sup> patient.--

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Furthermore, the new proteins ~~conducting~~ of Figure 1 - SEQ ID No. 1  
its mutants and variations, as well as parts thereof are placed under protection. <sup>a part of the present invention.</sup>

Especially <sup>available</sup> preferred partial sequences are the amino acids 78 to 200 (RGS) - SEQ  
ID No. 2, 343 - 396 (GSK 3b-binding domains) - SEQ ID. No. 3, 397 - 465 (b-  
catenine binding domains) - SEQ ID No. 4 and 783 - 833 (disheveled homology  
region) - SEQ ID No. 5. Partial sequences of the Adenomatosis Poliposis Coli  
(APC), which are characterized by the amino acid sequences 1464 - 1604, 1516 -  
1595, 1690 - 1778 and 1995 - 2083 as <sup>invention.</sup> ~~RGS domains~~ interaction sites, are also part  
of the ~~extent of the protection~~.

<sup>as are also</sup> Like <sup>the</sup> ~~analogous~~ cDNA sequences, especially the full cDNA  
sequence of the ~~conducting~~ (base pairs 1 - 2825) of Figure 2 - SEQ ID No. 6, as  
well as the partial sequences of the ~~conducting~~ of the nucleotide sequence 446 - 814  
(RGS gene section) - SEQ ID No. 7, of the nucleotide sequence 1241 - 1402 (gene  
section of GSK 3b-binding domains) - SEQ ID No. 8, 1403 - 1609 (gene section of  
the b-catenine binding domains) - SEQ ID No. 9 and of the nucleotide sequence  
2561 - 2713 (gene section of the disheveled homology region) - SEQ ID No. 10.

The invention is explained in greater detail by the following  
examples.

Conducting was identified by a yeast 2-hybrid screen as a  $\beta$ -catenine  
interaction partner. The complete cDNA sequence was subsequently isolated and  
sequenced. The derived amino acid sequence of conducting is shown in Figure 1.

The nucleotide sequence in Figure 2 and the gene comparison of the amino acid  
sequence and the nucleotide sequence is shown in Figure 3. <sup>shown at position 1-2825</sup> Conducting consists of  
840 amino acids and has a <sup>calculated</sup> molecular weight of 92.8 kDa. By a comparison of  
sequences, an RGS domain (amino acid 78 - 200) and a domain (amino acid 783 -

The RGS domains (shown in double underlining, the  
 $\beta$ -catenine binding domains (shown in single  
underlining) and the disheveled homology region.  
(bold letters) are emphasized.

with the sequence regions  
marked as in Fig. 1.

833, disheveled homology region), related to the protein disheveled, were identified (Figures 1 – 3). The GSK 3 $\beta$  and  $\beta$ -catenin binding domains (amino acids 343 – 396 to 397 – 465) were discovered by interaction studies in the 2-hybrid system (Figure 4). It was observed that these domains are sufficient and necessary for the binding to GSK 3 $\beta$  or to  $\beta$ -catenin (Figure 4). On the other hand, the RGS homology region and the disheveled homology region do not participate. The interaction of conductine with GSK 3 $\beta$  and  $\beta$ -catenin was also confirmed biochemically in co-immunoprecipitation experiments.

The effect of conductine on  $\beta$ -catenin was investigated in SW480 cells. In these cells, the tumor suppressor gene product APC is mutated, as a result of which there is an increase in the cytoplasmic and especially in the nuclear content of  $\beta$ -catenin. The introduction of conductine into these cells leads to a drastic breakdown of  $\beta$ -catenin, as a result of which the cells are depleted of cytoplasmic  $\beta$ -catenin and of  $\beta$ -catenin in the cell nucleus (Figure 4). This effect on the content of  $\beta$ -catenin is equal in intensity to that of not-mutated APC, from which it can be concluded that conductine also acts as a tumor suppressor by regulating  $\beta$ -catenin. Moreover, it was shown that conductine also inhibits the Wnt/wingless signal path in *Xenopus* embryos due to its effect on  $\beta$ -catenin.

Wnt/wingless are secreted proteins that counteract conductin function in various tissues.

Furthermore, it was noted that conductine interacts directly with APC. APC fragments of amino acids 1464 – 1604, 1516 – 1595, 1690 – 1778 and 1995 – 2083 were identified as interaction sites for conductine. In conductine, the binding to APC takes place over the RGS domains; this region is sufficient and necessary for the interaction. The other domains in conductine do not participate (Figure 4).



## Legends for the Figures

### Figure 1

#### Amino Acid Sequence of Conductine

The conductine cDNA codes a protein of 840 amino acids with a calculated molecular weight of 92.8 kDa. The RGS domains (double underlining), the  $\beta$ -catenine binding domains (simple underlining) and the disheveled homology region are emphasized by bold lettering.

### Figure 2

#### Nucleotide Sequence of Conductine at Position 1 – 2825

The sequence regions are marked as in Figure 1.

### Figure 3

#### Comparison of Amino Acid Sequence and Nucleotide Sequence of Conductine

### Figure 4

#### Analysis of the Interaction of Conductine and its Parts with $\beta$ -Catenine, APC and GSK 3 $\beta$

The conductine protein and derived partial pieces are shown diagrammatically. The RGS domains (RGS), the GSK 3 $\beta$ -binding domains (GSK BD) and the  $\beta$ -catenine binding sites ( $\beta$ -BD) are emphasized. The interaction with  $\beta$ -catenine with the APC fragments of amino acids 1464 – 1604 (APCfr.1) and 1516 – 1595 (APCfr. 2) and GSK 3 $\beta$  were investigated in the yeast 2-hybrid assay and quantified as galactosidase units. It can be seen that the binding of the  $\beta$ -catenine to the  $\beta$ -



catenine binding site is limited; the other parts of the protein do not contribute to this. The analysis furthermore shows the exclusive interaction of APC with the RGS domains of conductin~~g~~. Comparable results for the binding to the RGS domains were obtained with the APC fragments of amino acids 1690 – 1778 and 1995 – 2083. The breakdown of  $\beta$ -catenine into SW480 cells by conductin~~g~~ was analyzed after transient expression of the given proteins and immunofluorescence staining of  $\beta$ -catenine. Only partial pieces of conductin~~g~~, which bind to  $\beta$ -catenine, lead to this breakdown. The analysis finally shows the binding of GSK 3 $\beta$  to the GSK 3 $\beta$ -binding domains of conductin~~g~~.

5